EFFICACY REVIEW

Product: **Kaput® Rat and Mouse Bait**

Date: May 30, 2003

EPA File Symbol: 72500-A

DP Bar code: D284997

Chemical Code: 086002

Formulation: Bait Formulation
0.025% Warfarin

Purpose for Review: The purpose for this review is to determine if Kaput® Rat and Mouse Bait is efficacious as a rat or mouse control product.

MRID(s):

45662901 R. Sayre. 2001. Standard Norway Rat (*Rattus norvegicus*) Anticoagulant Bait Laboratory Test Method with Kaput® Rat and Mouse Bait (0.025% Warfarin). Genesis Laboratories, Inc. Unpublished Report. Study #01031. 77pp.

45662903 J. Mach. 2001. Standard Norway Rat (*Rattus norvegicus*) Anticoagulant Bait Laboratory Test Method with Kaput® Rat and Mouse Bait (0.025% Warfarin). Genesis Laboratories, Inc. Unpublished Report. Study #99046. 112pp.

45662904 J. Mach. 2001. Standard House Mouse (*Mus musculus*) Anticoagulant Bait Laboratory Test Method with Kaput® Rat and Mouse Bait (0.025% Warfarin). Genesis Laboratories, Inc. Unpublished Report. Study #99047. 106pp.

Good Laboratory Practices: Yes

Branch Supervisor: Meredith Laws, Branch Chief

Team Reviewer: John Hebert, Acting Product Manager -PM Team 04

IRB Reviewer: Geraldine R. McCann, Environmental Protection Specialist

BACKGROUND: SCIMETRICS, LTD. CORP. has applied for a new pesticide registration of a 0.025% warfarin product called Kaput® Rat and Mouse Bait; a grain bait for the control of rats and mice. Two of the efficacy tests were conducted according to the guidelines specified in the Standard Norway Rat Anticoagulant Dry Bait Laboratory Test Method. OPP Designation:

1.203 (8-15-80) and one was conducted in accordance with the Standard House Mouse Anticoagulant Dry Bait Laboratory Test Method. OPP Designation: 1.204 (8-15-80). This is a review for the three efficacy tests and a product label.

REVIEW OF DATA:

1. **45662901** R. Sayre. 2001. Standard Norway Rat (*Rattus norvegicus*) Anticoagulant Bait Laboratory Test Method with Kaput® Rat and Mouse Bait (0.025% Warfarin). Genesis Laboratories, Inc. Unpublished Report. Study #01031. 77pp.

DISCUSSION: This study was conducted to determine the efficacy of the Kaput® Rat and Mouse Bait (0.025% Warfarin) on male and female Wistar albino rats from Harlan Sprague Dawley, Inc. in Indianapolis, Indiana.

Rats arrived at the test facility August 8, 2001. The testing began September 5, 2001. The rats were held pretest for 27 days (7days quarantine, 13 holding days, and 7 days pretest acclimation to the test room). The test lasted 12 days. The last test animal died on day 10, and the control animals came off test on day 12 (three days early). The guidelines specify that Standard OPP diet be fed for the duration of the 15-day test with 5 days posttreatment. Even if the last test animal died on day 12, the control animals should have been fed for 5 days following the end of the test (which would have been until day 17).

The difference between the average pretest weights for the male and female rats should have been within 50 grams and the average difference in the weights pretest was 86.77 grams. This is not a significant difference, this criteria of the test guidelines were stretched a bit, but the results are acceptable and do not appear to have affected the outcome of the test. I find this weight difference acceptable, though not within the guidelines of 1.203, 2.1.

In the study report, there is no mention of a batch or reference number to the bait (to Kaput® Rat and Mouse Bait) that was formulated by Genesis Laboratories, Inc. on September 4, 2001 (page 10 of 77). This means we may not have a validated method associated with the bait for this study for enforcement purposes. This formulation has not been seen by the Technical Review Branch. We need batch or reference numbers and a validated analytical method associated with this formulation to consider this efficacy study.

Bait acceptance for the two treated groups combined was 33.4%. Paper plates were placed beneath the feeding area of the rats to catch any spilled bait or challenge diet. When weighing back the bait for bait acceptance, the spillage was added back into the animal dishes ("original feed cup"). Did the control animals have 2 test dishes/containers? I question the accuracy of this practice not to mention that the animals were fed out of these dishes and if the spillage was in the

cage or underneath it, it possibly has been urinated or defecated on. This violates the OPP guideline 1.203, 6.3. I need more clarification about this laboratory's practice of collecting spillage at this laboratory to determine if it affects the consumption.

Mortality of the test animals was 100%. No control animals died.
Results of the rat test are summarized below:

Table 1. Rep I - Rats on Kaput® Rat and Mouse Bait

Pretest Weights **Bait Consumption and Mortality**

Sex	Average Group Weight (g)	OPP Diet Consumed (g)	Kaput® Bait Consumed (g)	Total Bait Consumption (g)
M (20)	372.36	1448.3	739.5	2187.8
F (20)	292.21	**100% Mortality**		Percent **Kaput® Bait** Consumed **33.8%**
Total (40)	Group Difference 80.15			

Table 2. Rep II - Rats on Kaput® Rat and Mouse Bait

Pretest Weights **Bait Consumption and Mortality**

Sex	Average Group Weight (g)	OPP Diet Consumed (g)	Kaput® Bait Consumed (g)	Total Bait Consumption (g)
M (20)	386.3	1567.3	765.1	2332.4
F (20)	301.4	**100% Mortality**		Percent **Kaput® Bait** Consumed **32.8%**
Total (40)	Group Difference 84.9			

Table 3. Test III-Rats on OPP Challenge Diet

Pretest Weights **Bait Consumption and Mortality**

Sex	Average Group Weight (g)	OPP Diet Consumed (g)
M (20)	386.22	5610.1
F (20)	290.95	**0% Mortality**
Total (40)	Group Difference 95.27	

2. **45662903** J. Mach. 2001. Standard Norway Rat (*Rattus norvegicus*) Anticoagulant Bait Laboratory Test Method with Kaput® Rat and Mouse Bait (0.025% Warfarin). Genesis Laboratories, Inc. Unpublished Report. Study #99046. 112pp.

DISCUSSION: This is a review of a report which is an amended final report conducted to address a formatting change according to the Sponsor's direction. All changes are noted below (copied from page 9 of the study report):

1. "Guidelines do not address a method for test bait storage. By placing the test substance in the freezer, we were attempting to maintain the integrity of the active ingredient and limit a possible confounding factor (bait degradation)."

2. "Temperature and humidity were gauged by the National Research Council recommendation (1996) and not by the guidelines set by EPA."

3. "Raw data for this study was included as part of the final report."

This study was conducted at Genesis Laboratories, Inc. in Wellington, Colorado, to determine the efficacy of the Kaput® Rat and Mouse Bait (0.025% Warfarin) on male and female Wistar albino rats from Harlan Sprague Dawley, Inc. in Indianapolis, Indiana. Rats arrived at the test facility December 20, 1999. The 15-day test with 5 days posttreatment began December 27, 1999 and ended January 16, 2000, (21 days).

The bait was formulated October 12, 1999, at Genesis Laboratories, Inc. (page 52) and the assigned number associated with the bait on the formulation page was 99046/47. This number coordinates with the Genesis Laboratory study numbers for rats and mice. The Kaput bait was placed in high density plastic bags, and logged into the walk in freezer (99-TS-40) on site until testing. The explanation for storing the bait in the freezer is from the study report (page 10):

> "This study was conducted under the OPP guideline 1.204. The laboratory setting is intended to be a controlled environment to limit confounding factors. The guidelines do not address a method for test substance storage. By placing the test substance in the freezer, we were attempting to maintain the integrity of the active ingredient, and limit a possible confounding factor (bait degradation)."

This is not an acceptable practice. There are several reasons why the bait should not be frozen. Retail outlets or customers are not expected to freeze the product before sale or use. A bait is to be tested as it would be used by the end user. In this case, by placing the bait in the freezer, the integrity of the bait may be compromised by moisture from condensation inside the plastic from the freezing and thawing. Moisture may affect the concentration of the active ingredient and cause "spikes" of higher concentration on the bait.

The guidelines specify that the OPP rat and mouse challenge diet be stored in a freezer at -18 ° C to preserve the integrity of the corn oil used to formulate the diet and prevent the corn oil from becoming rancid.

Wood shavings should not be used (page 11) for bedding on animals planning to be used in tests that require a wire mesh bottom cage. Transferring the animals from the plush cage to a cold stark cage without acclimation is traumatic and requires time for acclimation. It is not clear if the shavings were left in the cages for the test process. I can't imagine that the rats were tested in cages with wood shavings and trying to recover spillage from beneath the cages amongst the wood shavings.

Bait acceptance for the two treated groups combined was 18.9 %. Paper plates were placed beneath the feeding area of the rats to catch any spilled bait or challenge diet. When weighing back the bait for bait acceptance, the spillage was added back into the animal dishes ("original feed cup") and reissued to the animal. I would question the accuracy of this practice not to mention that the animals were fed out of these dishes and if the spillage was in the cage or underneath it, it possibly has been urinated or defecated on. I need more clarification about this practice of collecting spillage at this laboratory to determine if it affects the consumption. When bait is spilled and falls beneath the cage, it is generally not available to the animal; however, the bait should be accounted for and added back to the equation, just not back to the test dish/container. Putting the spilled bait back into the test dish container would constitute a soiled dish and need to be replaced as specified in the guidelines (1.203, 6.3). The treated groups had 2 feed cups for bait and alternating cups to prevent positional bias. The control group had only 1 test dish/container. No posttreatment data is included with this submission.

The Harlan Teklad 8664 rodent diet was fed to the animals ad libitum during the holding, acclimation, and post-test periods (page 11). The rats are supposed to be fed the OPP rat and mouse challenge diet for post-test periods per OPP guideline 1.203, 8.2.

The males in the test weighed on average more that the 300 grams (_ = 337.3 grams) specified in the OPP guidelines (1.203, 2.1) and the females averaged between 150 and 300 (_ = 261.0 grams) which is acceptable. The difference between the average pretest weights for the male and female rats should have been within 50 grams and the average difference in the weights pretest was 76.3 grams. This is not significant enough to affect the outcome of the feeding test and I find this weight difference acceptable.

The guidelines call for the test room temperature to be within 20 to 25 °C and the actual temperature ranged from 16 to 26 °C. The range in temperature is acceptable. The guidelines specify the humidity in the test room to range between 50 and 55 % relative humidity. The humidity recorded in the test room at Genesis

Laboratories, Inc. was 20 to 25 %. This is not acceptable. Certainly some method of humidification can be accomplished.

Mortality in both treated groups was 100% for anticoagulant bait. The control group did not experience mortality. The last test animal died on day 15. Results of the rat test are summarized below:

Table 1. Rep I - Rats on Kaput® Rat and Mouse Bait
Pretest Weights Bait Consumption and Mortality

Sex	Average Group Weight (g)	OPP Diet Consumed (g)	Kaput® Bait Consumed (g)	Total Bait Consumption (g)
M (10)	338.6	2242.6	474.4	2720.0
F (10)	261.6	**100% Mortality**		Percent **Kaput® Bait** Consumed **17.4%**
Total (20)	Group Difference 77.0			

Table 2. Rep II - Rats on Kaput® Rat and Mouse Bait
Pretest Weights Bait Consumption and Mortality

Sex	Average Group Weight (g)	OPP Diet Consumed (g)	Kaput® Bait Consumed (g)	Total Bait Consumption (g)
M (10)	342.4	2108.4	539.5	2647.9
F (10)	275.7	**100% Mortality**		Percent **Kaput® Bait** Consumed **20.4%**
Total (20)	Group Difference 66.7			

Table 3. Test III-Rats on OPP Challenge Diet
Pretest Weights Bait Consumption and Mortality

Sex	Average Group Weight (g)	OPP Diet Consumed (g)
M (10)	330.9	7078.8
F (10)	263.8	**0% Mortality**
Total (20)	Group Difference 67.1	

3. **45662904** J. Mach. 2001. Standard House Mouse (*Mus musculus*) Anticoagulant Bait Laboratory Test Method with Kaput® Rat and Mouse Bait (0.025% Warfarin). Genesis Laboratories, Inc. Unpublished Report. #99047. 106 pp.

DISCUSSION: This study was conducted to determine the efficacy of Kaput® Rat and Mouse Bait on male and female mice. The report is an amended final report conducted to address a formatting change according to the Sponsor's direction. All changes are noted below (copied from page 9 of the study report (#99047):

1. "Guidelines do not address a method for test bait storage. By placing the test substance in the freezer, we were attempting to maintain the integrity of the active ingredient and limit a possible confounding factor (bait degradation)."

2. "Temperature and humidity were gauged by the National Research Council recommendation (1996) and not by the guidelines set by EPA."

3. "Raw data for this study was included as part of the final report."

This study was conducted at Genesis Laboratories, Inc. in Wellington, Colorado, to determine the efficacy of the Kaput® Rat and Mouse Bait (0.025% Warfarin) on laboratory mice from the Harlan Sprague Dawley; Indianapolis, Indiana. On October 25, 1999, Genesis Laboratories, Inc. received sixty-five laboratory house mice weighing between 14.6 and 30.5 grams. The mice were acclimated to the test room for 7 days. The 15-day test with 5 days posttreatment (per OPP guideline 1.204) began November 2, 1999, and ended November 16, 1999.

The housing for the caged mice was less than half the recommended size according to the OPP guidelines 1.204, 3.1. The recommended size is 2000 cm^2 and the size being used for this study is 972 cm^2. No shelters were mentioned in the report and there should be 2 shelters per 5 mice (OPP guidelines 1.204, 3.2).

The bait was formulated October 12, 1999, at Genesis Laboratories, Inc. (page 52) and the assigned number associated with the bait on the formulation page was 99046/47. This number coordinates with the Genesis Laboratory study numbers for rats and mice. The Kaput bait was placed in high density plastic bags, and logged into the walk in freezer (99-TS-40) on site until testing. The explanation for storing the bait in the freezer is from the study report (page 10):

> "This study was conducted under the OPP guideline 1.204. The laboratory setting is intended to be a controlled environment to limit confounding factors. The guidelines do not address a method for test substance storage. By placing the test substance in the freezer, we were attempting to maintain the integrity of the active ingredient, and limit a possible confounding factor (bait degradation)."

This is not an acceptable practice. There are several reasons why the bait should not be frozen. Retail outlets or customers are not expected to freeze the product before sale or use. A bait is to be tested as it would be used by the end user. In this case, by placing the bait in the freezer, the integrity of the bait may be compromised by moisture from condensation inside the plastic from the freezing and thawing. Moisture may affect the concentration of the active ingredient and cause "spikes" of higher concentration on the bait. Storage stability tests are needed to confirm the bait has not been altered by this practice.

The guidelines specify that the OPP rat and mouse challenge diet be stored in a freezer at -18 ° C to preserve the integrity of the corn oil used to formulate the diet and prevent the corn oil from becoming rancid (OPP guideline 1.204, 5.2.4).

Wood shavings should not be used for bedding on animals planning to be used in tests that require a wire mesh bottom cage. Transferring the animals from the plush cage to a cold stark cage without acclimation is traumatic and requires time for acclimation. It is not clear if the shavings were left in the cages for the test process. Nor was it explained what type of shelters the mice were given if any (which are called for in the OPP guidelines 1.204, 3.2).

The Harlan Teklad 8664 rodent diet was fed to the animals ad libitum during the holding, acclimation, and post-test periods (page 11). The mice were supposed to be fed the OPP rat and mouse challenge diet for post-test periods. This is another deviation from guideline procedure.

The males in the test weighed an average of _ = 23.3 grams and the females weighed an average of _ = 22.1 grams, which is acceptable. The difference between the average pretest weights for the male and female mice needed to be within 5 grams and the average difference in the weights pretest was 1.2 grams. This weight difference is acceptable per the OPP guidelines 1.204, 2.1.

The guidelines call for the test room temperature to be within 20 to 25 °C and the actual temperature ranged from 17 to 24 °C. The range in temperature is acceptable. The guidelines specify the humidity in the test room to range between 50 and 55 % relative humidity. The humidity recorded in the test room at Genesis Laboratories, Inc. was 16 to 26 %. During the acclimation period, the humidity was 11 % to 31 %. This is not acceptable. Some form of regulated humidification must be available for use in these test rooms.

For this test to be acceptable, a 33% toxic bait consumption was necessary. Bait acceptance for the two treated groups combined was 48.4%. This seems very high On page 102 of the study report, J. Mach explains a problem with 4 weights that were not recorded correctly in the Replicate II test group and the data was considered ambiguous and deleted. This would not have affected the combined acceptance.

"Food strainers" were used in the mouse dishes to help prevent spillage, but it was not mentioned if the design of the dishes kept the mice from nesting in them. Mice sleeping in the dishes has been known to change the weight of the bait. There was no mention of more than one test dish/container for control bait. OPP guidelines I.204, 6.3 states that "the control group is offered only the EPA rat and mouse challenge diet, which shall be presented in amounts and numbers of containers equivalent to those used for the test group." Paper plates were placed beneath the feeding area of the rats to catch any spilled bait or challenge diet. When weighing back the bait for bait acceptance, the spillage was added back into the animal dishes ("original feed cup"). I would question the accuracy of this practice not to mention that the animals were fed out of these dishes and if the spillage was in the cage or underneath it, it possibly has been urinated or defecated on. In some cases water may be on the plates from the water bottles and moisten the baits that have been spilled. There is no mention of drying the spillage before weighing it back. I need more clarification about this practice of collecting spillage at this laboratory to determine if it affects the consumption.

Another 90% mortality criteria was necessary for this test to be acceptable. Mortality of the test animals was 100%. The last test animal died on day 9, no control animals died. The guidelines clearly state that the test is to "continue with posttest feeding of the OPP rat and mouse challenge diet and observation of the surviving mice for a minimum of 5 days following the test period." (OPP guidelines I.204, 8.I). It appears from the data that the feeding stopped on Day 9 for the control mice. Results of the mouse test is summarized below:

Table 1. Replicate I on Kaput®
Pretest Weights **Bait Consumption and Mortality**

Sex	Average Group Weight (g)	OPP Diet Consumed (g)	Kaput® Bait Consumed (g)	Total Bait Consumption (g)
M (10)	20.8	127.9	107.2	235.1
F (10)	22.4	**100% Mortality**		Percent Kaput® Consumed **45.6%**
Total (20)	Group Difference 1.6			

Table 2. Replicate II on Kaput®

Pretest Weights **Bait Consumption and Mortality**

Sex	Average Group Weight (g)	OPP Diet Consumed (g)	Kaput® Bait Consumed (g)	Total Bait Consumption (g)
M (10)	24.8	119.9	125.8	245.7
F (10)	20.4	**100% Mortality**		Percent Kaput® Consumed **51.2%**
Total (20)	Group Difference 4.4			

Table 3. Mice on Control Bait

Pretest Weights **Bait Consumption and Mortality**

Sex	Average Group Weight (g)	OPP Diet Consumed (g)
M (10)	24.4	621.6
F (10)	23.5	**0 % Mortality**
Total (20)	Group Difference 0.90	

Efficacy Comments

1. The rat efficacy report attributed to R. Sayre (Study # 01031; MRID 45662901) did not indicate a batch number or a reference number to associate the bait used for the test to the bait analysis submitted separately. This study is upgradeable by providing a historical batch sheet which documents the composition of the test bait.

2. In the rat and mouse efficacy reports attributed to J. Mach (Study #'s 99046 and 99047, MRID 45662903 and 45662904), the study reports indicated that the OPP guidelines 1.203 and 1.204 were followed, respectfully. The study report states that a method for test bait storage wasn't mentioned. The freezing of bait before presentation reportedly was performed to limit a possible confounding factor (bait degradation). It is not an acceptable practice to freeze the test bait. This makes both studies marginally acceptable.

Efficacy Comments (cont.)

3. As freezing or even refrigeration of bait would not be a realistic requirement (or a likely practice) for a registered rodenticide bait product from the time that it is manufactured until it is used, we

believe that freezing the bait prior to its use added a confounding factor rather than removing one. Even with the freezing and the likely significant contribution of moisture loss to the data reported for bait "consumption", composite acceptance figures were low as were the individual acceptance results.

4. The OPP guidelines were not followed when the research laboratory chose to use the National Research Council recommendations for temperature and humidity instead of the OPP guidelines. This also makes both of these studies marginally acceptable.

5. In all three efficacy studies, the complete raw data package was not submitted or the studies were not completed. No posttest data was submitted for any of the three tests which suggests that the lab did not do the posttesting as stated in OPP guidelines 1.203 and 1.204. This makes both of these studies marginally acceptable.

6. If these unacceptable practices mentioned above are continued in the future, studies will be rejected.

Label Comments

1. Line up the decimal points on the Active Ingredient Statement.

   In the **ENVIRONMENTAL HAZARDS** section, please add a statement regarding secondary poisoning.

2. In number 3 under **DIRECTIONS FOR USE**, add a "d" to an to read in "Follow all application directions and **USE RESTRICTIONS**..."

3. Under **USE RESTRICTIONS**, delete the statement "similar manmade structures" because it is vague and ambiguous. Revise the statement to say: "This product may be used to control Norway rats, roof rats, and house mice in and around homes, industrial, commercial and public

buildings."

4. In the next sentence, add a "d" to **an** to read : "...(ships, trains, aircraft) and in a**nd** around related port..."

5. In that same paragraph under **USE RESTRICTIONS**, "This product may also be used in alleys." should read: "This product may be also be used in alleys in secured or otherwise immobilized bait stations."

6. In the **SELECTION OF TREATMENT AREAS** section: the phrase: "...in or beside burrows,..." suggests that this bait may be used in a field situation. Also, the following phrase **is** too vague and ambiguous and should be deleted: "...in corners and concealed places,..." This statement should read: "Generally, these areas are along walls by gnawed openings, in or beside burrows within 15 feet of a building or wall, between floors and walls, or in locations where rodents or their signs have been seen."

7. In the **APPLICATION DIRECTIONS** add "tamper resistant" before "bait station" in the **RATS** and **MICE** sections.

8. In the **APPLICATION DIRECTIONS**, revise the **RATS AND MICE** section to: "If reinfestation does occur, repeat treatment. Where a permanent source of infestation is present, establish permanent bait stations and replenish the bait as needed."

9. Two **STORAGE AND DISPOSAL** statements are needed: one for the container and one for pesticide disposal. Add a separate **STORAGE AND DISPOSAL** statement to say:

STORAGE AND DISPOSAL

Do not contaminated water, food, or feed by s6orgae or disposal.

Storage: Store in original container in a dry location inaccessible to children and pets.

Pesticide Disposal: Wastes resulting from the use of this product may be disposed of on site or at an approved waste disposal facility.

Container Disposal: Dispose of empty container in a sanitary landfill, or by incineration, or if allowed by state and local authorities, by burning. If burned, stay out of smoke.